SOME APPLICATIONS OF INSECT SEPARATION METHODS TO ENTOMOLOGY.

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In some entomological studies there is a need for methods that will separate insects parts from media such as soil, manure, etc. Peterson (1934) figures several devices such as the Lathrop and Nickels (1932) apparatus for concentrating pupae. Blueberry work in New Jersey (Beckwith 1941) met with certain difficulties in measuring the amount of infestation and it is possible that additional methods for the removal of worms from the berries might have facilitated the investigation. Similarly, flotation or sedimentation procedures might be applied to toxicity studies of minute insects or mites. Hamilton (1941) counted the live and dead red spiders on rose leaves.

Berlese (1921) described an apparatus for the separation of small animals from soil. This "Berlese Funnel" uses an alcohol flotation in an especially constructed elongate tube. Shirck (1930) described "an apparatus for separating eggs and young larvae of wireworms from field samples of soil by washing . . ." which utilizes a water flow and a series of screens. Davidson and Swan (1933) floated insects from pasture soil and skimmed them off.

The multiple complications arising as the result of accumulated foods held under conditions where there is an insufficiency of proper storage facilities, and the infestation problems that will develop if the use of certain insecticides is further curtailed because of reallocation for the war effort, will certainly lead to further insect control investigations. These problems will be undertaken with reduced personnel and appropriations. Entomologists may, therefore, be interested in an approach to insect studies that has been developed somewhat outside of their immediate field.

Work by the U. S. Food and Drug Administration in enforcing the Food, Drug, and Cosmetic Act has demonstrated that rather unusual methods may be employed to remove insects and insect parts from various food substances as a preliminary to their quantitative estimation. Tests have indicated that it is mechanically practical to remove small insects to a filter paper and if it is unnecessary to know whether the insects are living or dead, it may be possible to make population studies in that manner.

For several years the U. S. Food and Drug Administration has used mechanical screening, flotation, sedimentation, and solubility methods to remove insects from foods and drugs. Descriptions of the insect and rodent filth methods usually have

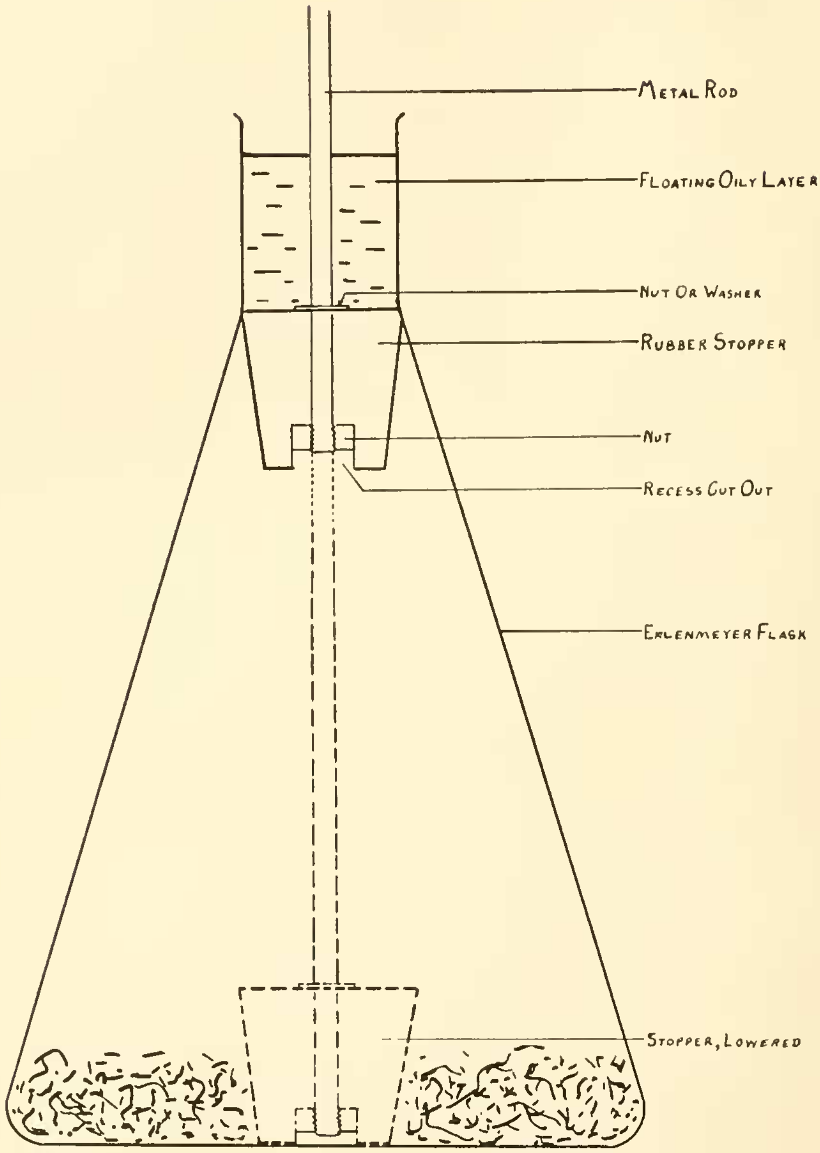

Figure 1. Wildman Trap Flask.

been mimeographed (U. S. Food and Drug Administration mimeographs) for distribution to parties interested in food examination. These methods utilize various procedures but usually are based upon one or more of three basic principles. (1) Solubility of the food or drug and insolubility of the insects. (2) Preferential wetting of insects or insect fragments by oily liquids whereby they acquire a lighter specific gravity which allows their separation by flotation methods. (3) Selective sedimentation in water or heavier-than-water liquids.

Some of these methods have been published (Wildman, 1932; Greene, 1935; Howard, 1935, 1937; Harris, 1941.) They have been used principally by food chemists and processors who are interested in the food purity situation. To a limited extent entomologists have utilized them as adjuncts to entomological surveys, as means of counting insects during control studies, or in life history work.

Many of the methods are based upon the use of a Wildman trap flask (Howard, 1937). This flask (see Figure 1) was devised by Mr. J. D. Wildman of the Microanalytical Division of the Food and Drug Administration for use in concentrating and recovering insect parts floating on liquids. It consists of a 1-liter or 2-liter Erlenmeyer flask into which is inserted a close-fitting rubber stopper supported on a stiff brass rod of 5/32″ to 6/32″ diameter and about 3″ longer than the depth of the flask. A rod of greater diameter is not desirable because of its greater displacement of liquid. The rod is threaded at the lower end and furnished with nuts and washers to hold the stopper in place. The lower nut and washer must be countersunk in the rubber to prevent the nut from striking the bottom of the flask. For some products (e. g., tomato products) a 2-liter flask is used, while for others (e. g., corn meal) the 1-liter size is preferred. In use, the food is put in the flask, water or other suitable liquid added, an oily liquid poured in and the mixture stirred with the rod. When the mixing is complete, water is added until the oily layer and some of the water rises into the neck of the flask. The insects are carried to the top and by raising the stopper the insect-bearing oil layer may be trapped off and decanted.

The best liquids to use in a Wildman flask for separation of insects and their remains from food or other substances depends on the character of the material being tested. Insects and insect fragments are often lighter than cereals and sometimes may be floated out in heavier-than-water liquids while the plant tissue settles out. Usually, however, they are extracted by a different procedure. With the exception of fly larvae, or maggots, insects and insect fragments can be wet with oils mixed into an aqueous mixture of a food and so floated up to the surface with the oil. In practice, because of several factors, this separation may be incomplete. It is difficult to wet all the insect material without creating a frothy emulsion of the plant material that will obscure a subsequent examination. Fragments may become trapped in or attached to a mass of plant material settling out. Droplets of oil often adhere to the sides of the trap flask and may hold insects there and so keep them from rising. To reduce some of these effects, the oil or gasoline is worked thoroughly into the water-cereal mixture, but with no "whipping" and as little inclusion of air as possible. Intermittent agitation is provided while the separation is taking place.

Some products cannot be extracted in water because too much of the food may rise with the "light filth." This rise of the food sometimes may be due to differences in specific gravity of parts of the food and the water, but often is caused by the formation of persistent emulsions, or by surface reactions that permit the food to be wet by an oil. To reduce floury emulsions, the extractions can be made in saturated salt solution. Sometimes caprylic alcohol or 95% ethyl alcohol can be used to break an emulsion. In general, when much bran or chaff is present it will float up with the oil when water or saturated salt solution is used and it is advisable to substitute a water-ethanol solution. (For some cereals a water-isopropyl alcohol solution may be used.) The alcohol not only soaks into bran but it is also less dense so that less plant tissue floats. Material trapped off in one Wildman trap may be transferred to another trap and re-washed to remove some of the plant material, or it may be transferred directly to a rapid-acting filter paper in a Buchner funnel where the liquid can be sucked off.

F lter papers can be so treated as to make the microscopic examination of the material deposited on them as simple as possible. Flour and bran can be cleared with mineral oil or chloral hydrate rendering insect fragments or excreta more readily visible. If mineral oil is used, the material on the filter must be air dried before the oil is added. Mineral oil is well suited to the microscopic examination of insects and insect fragments. If an excessive amount of starchy material is present, the paper may be completely cleared by gelatinizing it with chloral hydrate. The chloral leaves the pellet fragments soft but is extremely noxious to work with. It can be washed out of the filter after the clearing is complete and before the microscopic examination is made.

In order to facilitate quantitative determinations the filter paper can be ruled in fine parallel lines 6 mm. apart before it is used. The lines can be applied conveniently by means of a rubber stamp and pad. Waterproof India ink makes a permanent non-spreading line. If the filter paper is not ruled, it is necessary to place a wire grid over the paper to mark it off into smaller areas.

The ramifications and adaptations of these methods may prove to be limitless. Thus, by modifying the method for the recovery of insect fragments from tomato products (Howard, 1938 mimeograph), Smith (1942 mimeograph) found that he could float most of the tomato tissue and so recover any maggots and fly eggs by sedimentation. Smith (1942) by the use of castor oil in place of gasoline for the caterpillar separation obtained completely satisfactory results and secured residues freer from plant cellular material.

Welch (1940) described a radically different apparatus and reported that insect larvae could be separated from pecan pieces mechanically by means of a machine containing a moving inclined belt of ordinary window screen of about 16 mesh. The infested pecans are fed into the middle of the belt. The rough edged pecans move up with the belt but the worms roll down.

Blumberg (1939) has reported on and devised several procedures to determine insects in foods and drugs. He mentions work by Spicer and Price (1938) in which sodium citrate was used to dissolve cheese; Wilder and Joslyn (1937) on insects in tomato products; Butcher (1934) on flour contamination; and others. Blumberg's (1939) findings involve a sifting and digestion method of finding insect eggs, also a flotation method for insect eggs which uses a special separatory funnel, and the use of clove oil for insect excreta.

In one joint survey which included the Bureau of Entomology and Plant Quarantine, Bureau of Plant Industry, Food and Drug Administration, and others (Cotton, et al, 1941) the flotation tests and the flour-oil test were employed to determine the presence of insect contamination in flour.

Literature Cited.

Beckwith, C. S. 1941. Control of Cranberry Fruit Worm on Blueberries. Jour. Econ. Ent. *34*, (2): 169–171.

Berlese, A. 1921. Mezzo per separate gli artropodi raccolti col *Collettore Berlese* dalla terra caduto con essi. Redia *14*: 211–214.

Blumberg, B. L. (1929). Insect Infestation in Drugs and Flours with Special Reference to the Genus *Tribolium*. Pharm. D. dissertation. Columbia University, College of Pharmacy.

Butcher, C. H. 1934. The Microscopy of Food Products, Part 5, Flour. Food *3* (35): 415–417.

Cotton, R. T., et al. 1941. Insect Infestation and Its Effect on Quality of Flours. American Miller, Oct.: 34–39.

Davidson J. and Swan, D. C. (1933). A Method for Obtaining Samples of the Population of Collembola (Symphypleana) in Pastures. Bull. Ent. Res. *24*, part 3: 351–352.

Greene, W. S. 1935. A Method for the Detection of Filth in the Form of Insects and Other Extraneous Materials in Butter. Food Industries. *7* (9) 441.

Hamilton, C. C. 1941. Toxicity of Methyl Bromide to the Common Red Spider and to Greenhouse Roses. Jour. Econ. Ent. *34* (2): 232–237.

Harris, K. L. 1941. Foreign Matter in Corn Meal. Cereal Chemistry *18* (5): 655–661.

Howard, B. J. 1935. Corn Ear Worm in Tomato Products. Food Industries *7* (7): 321–2.

Howard, B. J. 1937. Fragment Count Methods for Tomato Products, Canning Age. *18* (9): 324–6.

Lathrop, F. H. and Nickels, C. B. 1932. The Biology and Control of the Blueberry Maggot in Washington County Maine. U. S. D. A. Tech. Bull. 275.

Peterson, Alvah. 1934. A Manual of Entomological Equipment and Methods. Part I. Edwards Brothers, Inc., Ann Arbor, Mich.

Shirck, F. H. 1930. A Soil-Washing Device for Use in Wireworm Investigations. Jour. Econ. Ent. *23* (6): 991–994.

Smith, F. R. 1942. Private communication.

Spicer, D. W. and Price, W. V. 1938. A Test for Extraneous Matter in Cheese. J. Dairy Sci. *21* (1): 1–5.

Welch, H. 1940. Food and Drug Administration communication.

Wilder, C. D. and Joslyn, M. A. 1937. Estimation of Worm and Insect Fragments in Tomato Products. J. Assoc. Official Agric. Chem. *20* (4): 648–655.

Wildman, J. D. 1932. A Simple Method for Separating Certain Insects from Food Products. Science *75* (1940): 168–9.

U. S. Food and Drug Administration Mimeographs:

A Method for the Determination of Extraneous Material in Butter. W. S. Greene. 11/22/35.

A Method for the Detection of Extraneous Matter in Cheese. W. S. Greene. 11/29/35.

Proposed Rapid Method for the Separation of Insects from Wheat Flour. Kenton L. Harris. 9/28/39.

Method for the Examination of Canned Field Peas (Cowpeas) for Insects. Kenton L. Harris. 7/28/39.

A Method for the Examination of Tea for Rodent and Insect Contamination. Kenton L. Harris. 2/21/41.

A Method for the Recovery of Filth from Rye Flour and Meal (Tentative). Kenton L. Harris. 12/18/41.

General Principles for the Study of Insect and Rodent Filth in Cereals. Kenton L. Harris. 10/12/41.

Methods for the Recovery of Extraneous Matter from Dried Eggs. Kenton L. Harris. 5/22/42.

Method for the Recovery of Filth from Corn Meal (Tentative). W. G. Helsel and Kenton L. Harris. 12/12/39.

Method for the Recovery of Filth and Foreign Matter from Wheat Meal. (Tentative.) W. G. Helsel and Kenton L. Harris. 3/27/40.

Recovery of Insects and Miscellaneous Filth from Apple Butter. F. Allen Hodges. 3/31/39.

Methods for the Examination of Brick Type Cheese for Mites. F. A. Hodges. 2/3/41.

Tanking of Blueberries for Removal of Maggots. B. J. Howard. Aug. 1927.

Method of Testing Cherries for Maggots. B. J. Howard. 9/25/37. (Originally issued in 1925 by the New York State Canners' Association).

Determination of Insect P rts in Tomato Products. B. J. Howard. Reissued 10/5/38.

Esti.nation of nsect Excreta in Flour. B. J. Howard. 1/7/39.

Method of Examination of Fig Paste for Insects. B. J. Howard and J. D. Wildman. 2/13/28. (Revised 12/19/39).

Testing Raspberries and Loganberries for Beetle Infestation. B. J. Howard and J. D. Wildman. May 1931.

Method for Determining Insect Excreta in Fig Paste. B. J. Howard and J. D. Wildman. Revised 12/19/39.

Method for the Recovery of Fly Eggs and Larvae in Tomato Products. Frank R. Smith 3/9/42.

Method for the Estimation of the Number of Insects in Canned Greens. J. D. Wildman. 5/21/37.

Examination of Candy for Filth. St. Louis Station, April 26, 1940.

Method for the Recovery of Filth from Flour. 9/12/38.

A NEW SPECIES OF CUTEREBRA FROM KANSAS. (*Diptera: Cuterebridae.*)

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The warble fly described below is a parasite of *Neotoma flavidianus osagensis* Blaiv. in the Vicinity of Fall River, Kans. It was first recovered by C. W. Hibbard, Department of Zoology, University of Kansas, Lawrence, Kans., who sent specimens to me for identification. It was later reared in some numbers by my friend R. H. Beamer, of the same institution. Because Dr. Beamer has a paper in preparation which describes the habits of this fly, it is necessary to publish the following description in advance of a larger work on the North American botflies which has been in the course of preparation for the past several years.

Cuterebra beameri, new species.

A medium-sized black species with infuscate wings.

Male.—Head black; vestiture black; frons three-fifths as wide as one eye; parafrontale and parafaciale with numerous minute punctures;